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Specific IgE for Fag e 3 Predicts Oral Buckwheat Food Challenge Test Results and Anaphylaxis: A Pilot Study

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Keywords

Buckwheat · Food allergy · Food hypersensitivity · Oral food challenge · Immunoglobulin E

Abstract

Background: Buckwheat (BW) is the source of a life-threatening allergen. Fag e 3-specific serum IgE (slgE) is more useful than BW-slgE for diagnosis; however, it is unknown whether Fag e 3-slgE can predict oral food challenge (OFC) results and anaphylaxis. This study aimed to clarify the efficacy of Fag e 3-slgE in predicting OFC results and anaphylaxis. **Methods:** We conducted a retrospective review of BW- and Fag e 3-slgE data obtained using the ImmunoCAP[®] assay system and fluorescent enzyme-linked immunosorbent assay from children who underwent OFC using 3,072 mg of BW protein between July 2006 and March 2014 at Sagami National Hospital, Kanagawa, Japan. **Results:** We analyzed 60 patients aged 1.9–13.4 years (median 6.0 years); 20 (33%) showed objective symptoms upon BW OFC. The patients without symptoms had significantly lower Fag e 3-slgE than those with non-anaphylactic ($p < 0.001$) and anaphylactic reactions to BW ($p = 0.004$). Fag e 3-slgE was the only test-

ed factor that significantly predicted positive OFC results (odds ratio 8.93, 95% confidence interval 3.10–25.73, $p < 0.001$) and OFC-induced anaphylaxis (2.67, 1.12–6.35, $p = 0.027$). We suggest that a threshold Fag e 3-slgE level of 18.0 kU_E/L has 95% probability of provoking a positive reaction to BW. **Conclusions:** Fag e 3-slgE predicted OFC results and OFC-induced anaphylaxis. We further emphasize paying careful attention to the risk of BW OFC-induced anaphylaxis.

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Introduction

Buckwheat (BW) has been widely consumed in Asian countries and is increasing in popularity in the USA and Europe [1, 2]. However, it can be a potent source of allergens and can cause life-threatening anaphylaxis. In Japan, BW is the fourth-most common cause of food-induced anaphylaxis [3–5]. The estimated prevalence of BW allergy in school-children in Japan is 0.22% [6]. More than

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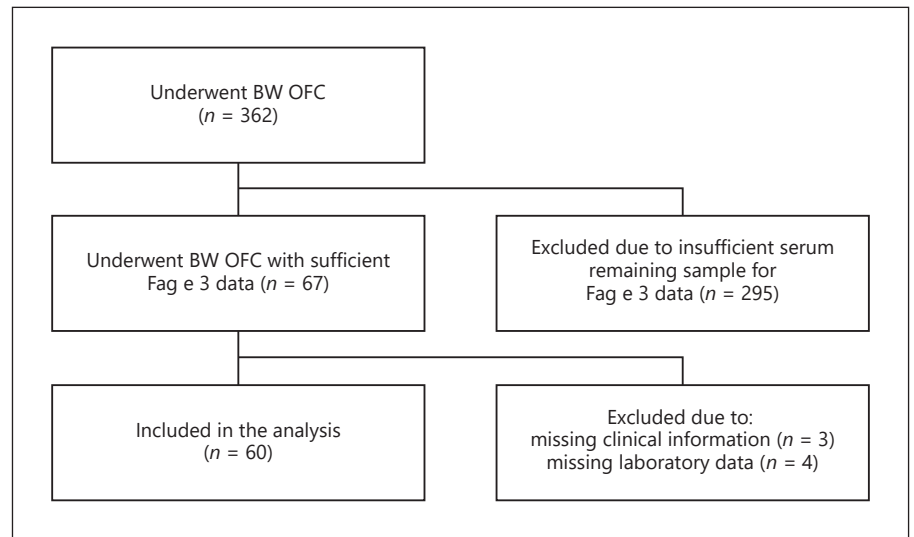


Fig. 1. Study participation.

half of challenge-positive patients for BW present anaphylaxis [4]. BW serum-specific IgE (sIgE) levels have been useful for diagnosing BW allergy [4]; however, Fag e 3 (7S globulin, vicilin fragment) is a novel candidate as a more useful BW allergen component for diagnosis [7] than BW alone. Currently, the most accurate diagnosis method for BW allergy is oral food challenge (OFC). Our study aimed to clarify the efficacy of Fag e 3-sIgE to predict OFC results.

Materials and Methods

Study Population and Enrollment

We retrospectively analyzed data from children who underwent an OFC with BW in Sagamihara National Hospital, Kanagawa, Japan, between July 2006 and April 2014. About 60% of patients were referred from primary care clinics, and the rest were admitted directly without referral. Patients who were allergic or suspected to be allergic to BW were diagnosed based on the presence of a history of immediate reaction to BW or past or current atopic dermatitis with positive BW-sIgE. We enrolled only those undergoing their first BW OFC and did not have missing clinical or laboratory data.

Oral Food Challenge

In total, 64 g of BW noodles containing 3,072 mg of BW protein were used for OFC as previously described [4] (based on the 2017 Japanese Pediatric Guidelines for Food Allergy [8]). Treatments for provoked reaction were based on the European Academy of Allergy and Clinical Immunology's food allergy and anaphylaxis guidelines [9]. Anaphylaxis was defined according to World Allergy Organization Anaphylaxis Guidelines [10], and severity of symptoms was defined according to Japanese anaphylaxis guidelines [8, 11].

Laboratory Data

We assessed BW-sIgE within 180 days of the OFC using the ImmunoCAP® assay system (Thermo Fisher Scientific/Phadia, Uppsala, Sweden). Fag e 3-sIgE was analyzed using stored serum samples via a fluorescent enzyme-linked immunosorbent assay (ELISA) at Kyoto University using previously described methods [7]. The serum sample was stored at -80°C for a median of 2.5 years (range 0.5–8.2 years) before analysis. These stored conditions did not affect the stability of sIgE [12–14].

Statistical Analysis

We expressed data as median values and ranges, used the Mann-Whitney U or the Fisher exact test for statistical comparisons, as appropriate, and considered p values of <0.05 as statistically significant. We performed univariate and multivariate analyses using logistic regression. To create probability curves, we used regression analysis after logarithmic transformation of sIgE values, as previously published [15]. We used SPSS v24.0 (IBM Corp., Armonk, NY, USA) to perform all statistical analyses.

Ethical Considerations

In accordance with the Declaration of Helsinki, the study design and risks of anaphylaxis during the OFC were fully explained to patients and their guardians, both orally and in writing, and written informed consent was obtained from all participants for the OFC and publication of the data. All patient data were anonymized prior to analysis. We obtained approval from the institutional review board of Sagamihara National Hospital.

Results

Patient Background

In total, 362 patients underwent an OFC (Fig. 1). We excluded 295 patients owing to insufficient serum sam-

Table 1. Clinical and demographic characteristics of patients

OFC results	No symptoms (<i>n</i> = 40)	Reactive		<i>p</i> value ^a
		non-anaphylactic (<i>n</i> = 13)	anaphylactic (<i>n</i> = 7)	
Male sex	30 (75.0)	9 (69.2)	4 (57.1)	0.545
Age, years	5.9 (1.9–13.4)	7.4 (3.2–12.3)	7.4 (2.7–10.6)	0.259
A history of				
An immediate reaction to BW	4 (10.0)	3 (23.1)	3 (42.9)	0.070
Anaphylaxis to BW	0 (0.0)	1 (7.7%)	0 (0.0)	0.333
Atopic dermatitis, current	18 (45.0)	5 (38.5)	4 (57.1)	>0.999
Bronchial asthma, current	8 (20.0)	5 (38.5)	1 (14.3)	0.519
BW-sIgE, kU _A /L	2.8 (0.76–25.9)	3.8 (0.4–15.2)	2.7 (0.15–12.6)	0.913
Fag e 3-sIgE, kU _E /L	0.09 (0.06–8.3)	4.1 (0.09–23.2)	4.3 (0.09–42.0)	<0.001
Total IgE, IU/mL	1,205 (2–17,100)	842 (113–9,510)	745 (26–1,930)	0.052

Values are expressed as *n* (%) or median (range). BW, buckwheat; sIgE, specific immunoglobulin E.

^a Represents the statistical significance between negative cases and reactive cases. No significant difference among the 3 groups (no symptoms, non-anaphylactic, and anaphylactic) was seen without Fag e 3 sIgE (*p* < 0.001).

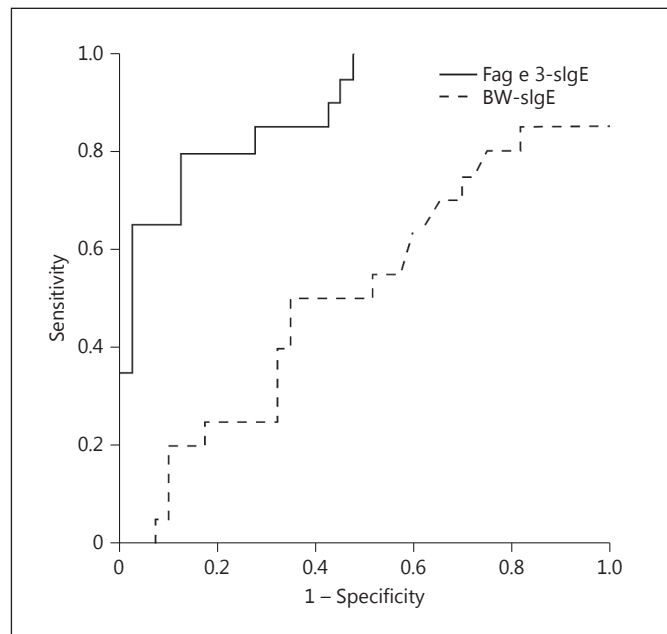


Fig. 2. ROC curve analysis to diagnose buckwheat allergy. BW, buckwheat; ROC, receiver-operating characteristic; sIgE, specific IgE.

ples for sIgE tests, 3 owing to a lack of clinical information, and 4 owing to missing laboratory data. The remaining 60 patients with suspected or definitive BW allergies were 1.9–13.4 years of age (median 6.0 years; Table 1), 10 of whom had a history of reactions to BW, and 1 with a

Table 2. Diagnostic performance of BW- and Fag e 3-sIgE

	BW-sIgE	Fag e 3-sIgE
AUC	0.509	0.893
95% CI	0.348–0.669	0.808–0.977
Optimal cut-off value, kU _E /L	3.8	0.3
Sensitivity, %	50.0	80.0
Specificity, %	65.0	87.3

AUC, area under the curve; BW, buckwheat; CI, confidence interval; sIgE, specific immunoglobulin E.

history of anaphylaxis due to BW. Median BW- and Fag e 3-sIgE levels were 2.8 kU_A/L (range 0.15–25.9) and 0.14 kU_E/L (range 0.06–42.0), respectively.

OFC Results

Twenty patients (33%) reacted, all with objective symptoms. Skin reactions were most common (15 [75%]), followed by respiratory (12 [60%]), gastrointestinal (11 [55%]), neurological (2 [10%]), and cardiovascular (1 [5%]) symptoms. We observed anaphylactic reactions in 7 patients (35%). Moderate and severe symptoms, as defined in Japanese Guidelines for Food Allergy [8], were present in 9 (45%) and 5 patients (25%), respectively.

Fourteen patients (70%) received treatment. Oral antihistamine was the most frequent treatment, adminis-

Table 3. Univariate and multivariate analyses of factors related to positive OFC results

Risk factors for positive OFC results (<i>n</i> = 60)	Crude OR (95% CI)	<i>p</i> value	Adjusted OR ^a (95% CI)	<i>p</i> value
BW-sIgE (10-fold increments)	0.800 (0.223–2.878)	0.733	1.026 (0.269–3.924)	0.970
Fag e 3-sIgE (10-fold increments)	8.930 (3.099–25.729)	<0.001	8.933 (2.927–27.265)	<0.001
History of an immediate reaction to BW	3.857 (0.944–15.763)	0.060	–	

BW, buckwheat; sIgE, specific immunoglobulin E; CI, confidence interval; OFC, oral food challenge; OR, odds ratio.

^a Adjusted according to a history of an immediate reaction to BW.

Table 4. Univariate and multivariate analyses of factors related to OFC-induced anaphylaxis

Risk factors for OFC-induced anaphylaxis (<i>n</i> = 60)	Crude OR (95% CI)	<i>p</i> value	Adjusted OR ^a (95% CI)	<i>p</i> value
BW-sIgE (10-fold increments)	0.62 (0.096–4.02)	0.617	0.89 (0.13–6.09)	0.903
Fag e 3-sIgE (10-fold increments)	2.67 (1.12–6.35)	0.027	2.33 (0.96–5.66)	0.061
History of an immediate reaction to BW	4.93 (0.91–26.85)	0.065	–	

BW, buckwheat; sIgE, specific immunoglobulin E; CI, confidence interval; OFC, oral food challenge; OR, odds ratio.

^a Adjusted according to a history of an immediate reaction to BW.

tered in 13 patients (65%). β_2 stimulant inhalation was administered to 11 patients (55%), intravenous steroids to 6 (30%), and intramuscular adrenaline injections to 2 (10%). Of 7 patients who showed anaphylaxis, 5 did not need adrenaline injections because of a rapid improvement in the respiratory symptoms following β_2 stimulant inhalation (*n* = 4), or improvement in moderate skin symptoms and repeated vomiting after the administration of intravenous antihistamines and steroids.

Diagnostic Performance of BW and Fag e 3-sIgE

The diagnostic performance of BW- and Fag e 3-sIgE was compared by calculating their respective area under the curve (AUC) and optimal cut-off values. The AUC for BW and Fag e 3-sIgE was 0.509 and 0.893, respectively (Fig. 2; Table 2). The optimal cut-off values were 3.8 kU_A/L and 0.3 kU_E/L, respectively.

Risk Factors for Positive Challenge

Patients with positive OFC results more frequently had higher Fag e 3-sIgE (10-fold increments; odds ratio [OR] 8.93, 95% confidence interval [CI] 3.10–25.73, *p* < 0.001) (Table 3). There was no significant difference be-

tween BW-sIgE and positive OFC results. There was also no significant difference between a history of an immediate reaction to BW and positive OFC. In a multivariate analysis adjusted for the presence of immediate reactions [4], Fag e 3-sIgE (10-fold increments, adjusted OR 8.93, 95% CI 2.93–27.27, *p* < 0.001) remained a significant factor for positive OFC results. In a univariate analysis, Fag e 3-sIgE was the only significant factor for OFC-induced anaphylaxis (10-fold increments, OR 2.67, 95% CI 1.12–6.35, *p* = 0.027) (Table 4), although this was nonsignificant in the multivariate analysis adjusted for immediate reaction.

Fag e 3-sIgE and Symptoms during OFC

Patients without symptoms had significantly lower Fag e 3-sIgE than those with non-anaphylactic (*p* < 0.001) or anaphylactic symptoms to BW (*p* = 0.004) (Fig. 3). Among challenge-positive patients (*n* = 20), those with gastrointestinal symptoms had higher Fag e 3-sIgE levels than those without such symptoms (*p* = 0.038). Although similar trends were observed for skin reactions and anaphylaxis with the Fag e 3-sIgE concentration, these were not statistically significant.

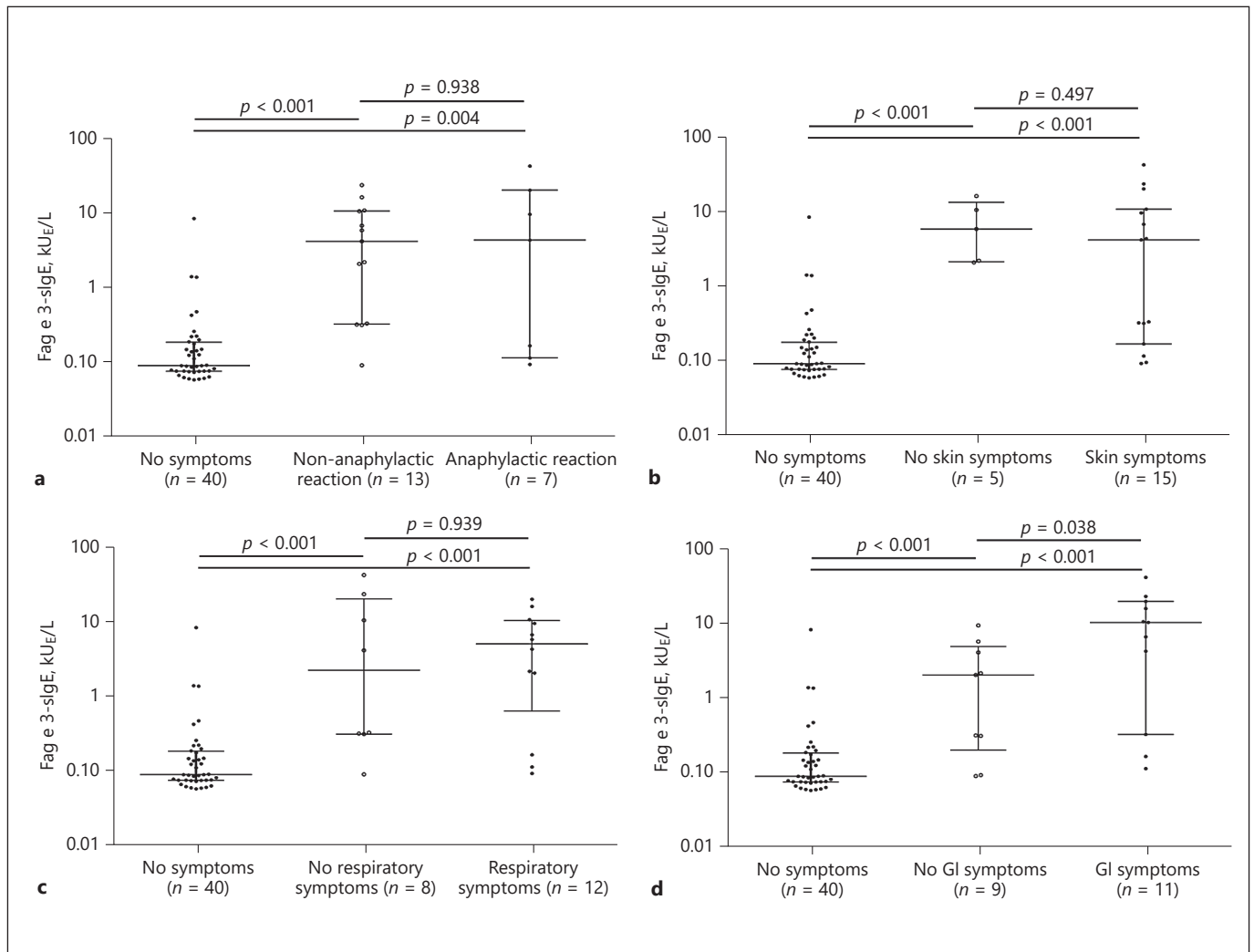


Fig. 3. Fag e 3-sIgE and symptoms during oral food challenge (OFC). **a** Fag e 3-sIgE among patients with no symptoms, non-anaphylactic reactions, and anaphylactic reactions during OFC. **b-d** Fag e 3-sIgE among patients with no symptoms; patients

showing symptoms other than skin, respiratory, and gastrointestinal (GI) symptoms; and patients with skin, respiratory, and GI symptoms, respectively, during OFC. Median and interquartile range are indicated by horizontal lines.

Probability of Failed Challenge and Anaphylaxis during OFC

Figure 4 presents fitted predicted probability curves for positive challenge and OFC-induced anaphylaxis at a given Fag e 3-sIgE concentration. We suggest that a threshold Fag e 3-sIgE concentration of 18.0 kU_E/L would have an approximately 95% probability of inducing any reaction to BW (Table 5). We were unable to calculate the sIgE concentration level at which approximately 95% of the patients are predicted to experience anaphylaxis (the 95% predictive decision point). The 50% predictive decision point for Fag e 3-sIgE to anaphylaxis was 75.4 kU_E/L.

Discussion

This was the first study to calculate the 95% predictive decision point for Fag e 3-sIgE. Although the usefulness of Fag e 3-sIgE has been reported, these studies included patients with a convincing history [6], whereas we included only OFC-proven BW-allergic and nonallergic patients. Consistent with a previous report [4], we observed frequent anaphylactic reactions and severe symptoms. Furthermore, we were able to estimate the risk of OFC-induced anaphylaxis, which clinicians should be aware of during OFC.

Table 5. Clinical efficacy of Fag e 3-sIgE for predicting a positive oral food challenge to buckwheat

Probability	50% pred. probability	90% pred. probability	95% pred. probability
Fag e 3-sIgE, kU _E /L	0.8	8.2	18.0
95% CI	0.4 to 3.0	2.4 to 200.0	-4.3 to 871.0

CI, confidential interval; sIgE, specific immunoglobulin E; pred., predicted.

BW OFC may induce severe reactions [4]. Therefore, knowing the probability for a reaction and anaphylaxis may help assess the risk of a positive OFC result and OFC-induced anaphylaxis prior to the challenge. Generally, many patients need BW OFC because BW-sIgE can only roughly predict OFC results. However, similar to a previous study [6], Fag e 3-sIgE was more useful than BW-sIgE for predicting positive reactions. It was also useful for predicting OFC results. This suggests that OFC with BW in patients with Fag e 3-sIgE >18 kU_E/L should be avoided. These results were derived using ELISA. Probability curves of different assays cannot be applied interchangeably [16]; therefore, it is unknown whether other methods would achieve 95% predictive decision points similar to those obtained for Fag e 3-sIgE.

In some foods, including those with minor allergens, 95% predictive decision points were reported [17, 18]. Component-resolved diagnosis (CRD) improves the accuracy of diagnosing IgE-mediated food allergy [19]. In some allergenic foods, like wheat and peanut, CRD (ω -5 gliadin, Ara h 2) provided a more precise diagnostic performance than crude food allergen-sIgE does, and only CRD showed 95% predictive decision points [20, 21]. Although BW-sIgE is useful [4], the 95% predictive decision points were not calculated. Our study also revealed that Fag 3e 3, a component of BW, is more useful than crude BW.

There were some limitations to our study. First, the OFCs were not double-blinded, placebo-controlled challenges. However, all OFC-positive patients had objective symptoms, including anaphylaxis; therefore, this should not actually affect the conclusions. Second, we performed a skin prick test (SPT) for only a few patients; combining the SPT and Fag e 3-sIgE may improve diagnostic performance. Moreover, we could not compare Fag e 3-sIgE and the SPT. Fag e 3-sIgE is currently commercially unavailable, and the SPT test is cheaper and faster. Accordingly, there is a need for further study to compare the two. Currently, the commercial availability of Fag e 3-sIgE is limited; therefore, its use in clinical settings is also limited.

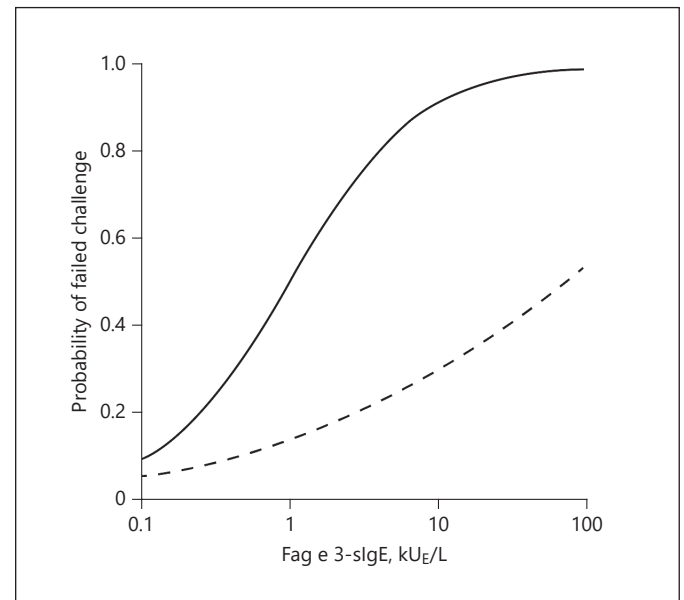


Fig. 4. Probability curves representing positive outcomes of oral food challenge (OFC) and anaphylaxis during OFC at a given Fag e 3-sIgE in 60 patients. The solid line represents the probability of an objective reaction ($n = 20$). The dotted line represents the probability of an anaphylactic reaction ($n = 7$).

Finally, there was a potential selection bias in our study. For example, patients with a strong history of anaphylaxis might avoid OFC. We excluded many patients with missing Fag e 3-sIgE data and included only 60 with BW OFC, primarily because many patients had an insufficient residual serum sample. The differences in Fag e 3-sIgE concentrations between patients with and without gastrointestinal symptoms, but not between those with other symptoms and anaphylaxis, may have originated from the small sample size. Larger prospective studies are needed.

In conclusion, Fag e 3-sIgE predicted OFC results and OFC-induced anaphylaxis. We further emphasize paying careful attention to the risk of BW OFC-induced anaphylaxis.

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Disclosure Statement

M. Ebisawa is on the DBV Technologies Scientific Advisory Board, and received lecture fees from Pfizer and Siemens. The remaining authors declare no relevant conflicts of interest.

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